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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/735,461	12/11/2003	Michael P. Czech	UMY-055	3119

959 7590 02/22/2007

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EXAMINER	
SCHNIZER, RICHARD A	
ART UNIT	PAPER NUMBER
1635	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	02/22/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/735,461	CZECH ET AL.	
	Examiner	Art Unit	
	Richard Schnizer, Ph. D.	1635	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 December 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *27 and 38-85* is/are pending in the application.

4a) Of the above claim(s) *60-78 and 80* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *27, 38-59, 79, and 81-85* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

An amendment was received on 12/12/06.

Claims 27 and 38-85 are pending in the application. Claims 60-78 and 80 stand withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 3/22/06.

Claims 27, 38-59, 79, and 81-85 are under consideration in this Action. Claim 81 is considered only to the extent that it depends from claim 79.

Rejections Withdrawn

Applicants amendments overcame the rejection of claims 52-55 under 35 U.S.C. 112, second paragraph.

The rejection of claims 38-42, and 45 under 35 U.S.C. 102(a) as being anticipated by Jiang et al (Proc. Nat. Acad. Sci. USA 100(13):7569-7574, 2003) is overcome by the Declarations under 37 CFR 1.132 regarding inventorship of the claimed invention.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 27, 38-59, 79, and 81-85 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods of identifying a

gene that affects glucose transport by contacting an isolated adipocyte with an siRNA targeted against the gene to form a mixture, electroporating the mixture, culturing the cell in vitro, and assaying glucose transport in vitro, does not reasonably provide enablement for such methods performed in vivo. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The claims are directed to methods of identifying a gene that affects glucose transport by contacting an adipocyte with an siRNA targeted against the gene to form a mixture, electroporating the mixture, culturing the cell, and assaying glucose transport. It is not clear that the limitation "culturing the cell" limits the invention to methods performed in vitro, so the broadest reasonable interpretation of the claims includes embodiments in which the methods are carried out on adipocytes in vivo.

The specification as filed provides no guidance as to how to perform the method in vivo, in particular how to measure glucose uptake or GLUT4 translocation in vivo while distinguishing electroporated cells from non-electroporated cells, such that one of skill could draw valid conclusions from any data obtained. The prior art of record provides no guidance in this regard, and such an assay was not routinely performed in the art at the time of the invention. As a result, one of skill in the art would have to perform undue experimentation in order to practice the invention as broadly claimed. This rejection could be overcome by requiring that the recited adipocytes must be "isolated".

Response to Arguments

Applicant's arguments filed 12/12/06 have been fully considered but they are not persuasive. Applicant requests withdrawal of the rejection in view of the amendment requiring "contacting in vitro an adipocyte" with an siRNA. This is unpersuasive because the claims as amended still embrace reimplantation of transfected adipocytes into an organism, and subsequent assay therein. This procedure is not enabled for the reasons set forth in the rejection of record. The rejection could be overcome by requiring that the recited adipocytes must be "isolated".

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 27, 44-48, 50, 51, 56-59, 79, and 81-83 are rejected under 35 U.S.C. 103(a) as being unpatentable over Al-Hasani et al (J. Biol. Chem. 273(28): 17504-17510, 1998) in view of Clancy et al (US 20030087259).

Al Hasani taught methods of studying genes related to glucose transport. Specifically, Al-Hasani investigated the relationship between the GTPase dynamin and endocytosis of the GLUT4 glucose transporter in cultured rat adipocytes. Adipocytes were transfected with a construct for expressing an easily detectable (HA)-tagged GLUT4, and then with either constructs for over-expression of either wild type dynamin

or a GTPase-negative mutant of dynamin. The effects of these dynamins on (HA)-tagged GLUT4 endocytosis after insulin treatment was measured. See abstract, paragraph bridging pages 17505 and 17506, Fig. 2 on page 17506, and Fig. 3 on page 17507.

Al Hasani did not teach the use of siRNA.

Clancy taught that the activity of a polypeptide in a cell can be controlled by several alternative means including the use of negative mutants of the protein and the use of antisense or siRNA directed at the mRNA encoding the protein. See summary of invention paragraph 9, detailed description paragraph 234, and claim 21.

It would have been obvious to one of ordinary skill in the art at the time of the invention to use siRNA directed against dynamin to assess its role in the endocytosis of GLUT4. For example, one could have used anti-dynamin siRNA to down-regulate wild type dynamin activity instead using the negative dynamin mutant. This experiment would result in down regulation of the endogenous dynamin (as required by instant claim 59) and the exogenous dynamin expressed from the expression construct (as required by instant claim 58). Further, one of ordinary skill in the art appreciates that the effects of the negative dynamin mutant could be confirmed by reversing them through the use of siRNA directed against the mutant. It would have been obvious to deliver the siRNA by electroporation because Al-Hasani demonstrated that this method was suitable for delivering nucleic acids to adipocytes.

Regarding claims 44-46, the temperature of electroporation, and the time between electroporation and assay, are considered to be variables that are routinely optimized by those of ordinary skill in the art, and so are considered to be obvious.

Claims 53-55 are included in the rejection because these claims, which require increased siRNA stability, or increased or decreased siRNA activity, recite no standard against which to compare stability or activity. One of ordinary skill in the art possesses the ability to modify a given siRNA to have greater or lesser activity and stability by incorporation of a greater or lesser number of modified bases. So, any given siRNA has greater or lesser activity than a differently modified one. In the absence of any standard of comparison these limitations carry no weight.

Claim 56 is included in the rejection because those of ordinary skill in the art appreciate that glucose metabolism is important in a variety of human diseases including diabetes. As a result it would be obvious to perform similar experiments in human cells.

Regarding claims 82 and 83, the concentrations of cells and siRNAs in the electroporation mixture are considered to be result-effective variables that are routinely optimized by those of ordinary skill in the art. Note that Al Hasani electroporated 200 microliters of cells at a concentration of 5-6 $X10^6$ cells per ml, i.e. about 1 million cells.

Claims 38-43, 84, and 85 are rejected under 35 U.S.C. 103(a) as being unpatentable over Al-Hasani et al (J. Biol. Chem. 273(28): 17504-17510, 1998) and Clancy et al (US 20030087259) as applied to claims 27, 44-48, 50, 51, 56-59, 79, and

81-83 above, and further in view of Paquereau et al (Anal. Biochem. 204(1): 147-151, 1992).

The teachings of Al-Hasani and Clancy are summarized above and can be combined to render obvious methods of identifying a gene that affects glucose transport by assaying insulin-mediated GLUT4 translocation in the presence or absence of dynamin, wherein dynamin concentration is modulated through siRNA delivered by electroporation at a potential of 0.2kV (see page 17505, column 1, second full paragraph).

The references are silent as to the capacitance setting for use in electroporation.

Paquereau taught a method of delivering nucleic acids to mammalian cells by electroporation using a potential of 0.15-0.2 kV and a capacitance of 960 micro F. These conditions minimized cell damage and increased cell survival. See abstract.

It would have been obvious to one of ordinary skill in the art at the time of the invention to optimize the electrical potential and capacitance used in the electroporation of the cells of Al-Hasani because it was recognized in the art that these variables could affect the amount of cell damage caused by electroporation, as well as cellular survival after electroporation. In so doing, one of ordinary skill would have noted that Al-Hasani used a voltage in the range used by Paquereau, and so would have been motivated to use a capacitance in the range used by Paquereau with the reasonable expectation of obtaining minimal cellular damage and improved cellular survival. Note that Paquereau used the exact capacitance required by instant claim 43.

Claim 49 is are rejected under 35 U.S.C. 103(a) as being unpatentable over Al-Hasani et al (J. Biol. Chem. 273(28): 17504-17510, 1998) and Clancy et al (US 20030087259) as applied to claims 27, 44-48, 50, 51, 53-59, 79, and 81 above, and further in view of Standaert et al (J. Biol. Chem. 272(48): 30075-30082, 1997).

The teachings of Al-Hasani and Clancy are summarized above and can be combined to render obvious methods of identifying a gene that affects glucose transport by assaying insulin-mediated GLUT4 translocation in the presence or absence of dynamin, wherein dynamin concentration is modulated through siRNA delivered by electroporation.

The references do not teach an assay of glucose uptake.

Standaert taught method of studying the effect of a gene expression of protein kinase C zeta (PKC-zeta) on glucose transport. Assays included measurement of GLUT4 translocation as well as glucose uptake. See abstract, paragraphs bridging pages 30078 to 30080, and Figs 7 and 8 on page 30079.

It would have been obvious to one of ordinary skill in the art at the time of the invention to extend the studies of Al-Hasani to studies of glucose uptake. One of ordinary skill in the art, interested in the effects of genes on glucose transport, would have realized that GLUT4 translocation and GLUT4 transport activity can both be used as measures of the effect of a gene product on glucose transport, and would have been motivated to use either one. However, one would have been particularly motivated to assay glucose uptake directly given that is the actual function of GLUT4, and so would

provide a more accurate representation of the effects of the gene product on glucose transport.

Claims 52-55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Al-Hasani et al (J. Biol. Chem. 273(28): 17504-17510, 1998) and Clancy et al (US 20030087259) as applied to claims 27, 44-48, 50, 51, 53-59, 79, and 81 above, and further in view of McSwiggen et al (US Patent 7,022,828).

The teachings of Al-Hasani and Clancy are summarized above and can be combined to render obvious methods of identifying a gene that affects glucose transport by assaying insulin-mediated GLUT4 translocation in the presence or absence of dynamin, wherein dynamin concentration is modulated through siRNA delivered by electroporation.

The references do not teach siRNA derivatives.

McSwiggen taught methods of inhibiting gene expression using siRNA, and taught that the stability of siRNA molecules could be enhanced through the use of modified bases. See and column 25, lines 58-67 claim 1.

It would have been obvious to one of ordinary skill in the art at the time of the invention to use modified siRNA oligonucleotides in the invention of Al-Hasani as modified by Clancy. One would have been motivated to do so in order to enhance the function of the oligonucleotides, as taught by McSwiggen.

Response to Arguments

Applicant's arguments filed 12/12/06 have been fully considered but they are not persuasive.

Applicant addresses the obviousness rejections at pages 13-21 of the response. Applicant argues that there is insufficient motivation to combine the Al Hasani and Clancy references, and further that one of ordinary skill would have had no reasonable expectation of success if the references were combined. At page 15 of the response, Applicant asserts that there is nothing in the cited art to lead one of ordinary skill to select siRNA from the list of gene blocking compounds disclosed by Clancy. This is unpersuasive because these gene blocking techniques are considered to be art-recognized equivalents, in view of the disclosure of Clancy. One of ordinary skill, aware of the Clancy reference, appreciates that gene expression can be inhibited by a variety of techniques, and that selecting a particular technique is simply a matter of design choice. MPEP 2144.06 indicates that when it is recognized in the art that elements of an invention can be substituted, one for the other, while retaining essential function, such elements are art-recognized equivalents. An express suggestion to substitute one equivalent process for another is not necessary to render such substitution obvious. Applicant's argument that Clancy is unrelated to glucose transport is unpersuasive because Clancy was not relied upon to teach glucose transport. Clancy was relied upon to demonstrate the level of knowledge of those of ordinary skill regarding the various means available to negatively regulate gene expression or protein function.

At page 16, Applicant argues that even if the references are combined, one of ordinary skill would have had no reasonable expectation of success. Applicant relies for support on the instant specification at page 1, lines 23-24, and on Walters and Jelinek (2002) (hereinafter Walters), and Weil et al (2002).

The specification at page 1, lines 23 and 24 indicates that no method has been reliably able to achieve specific ablation of gene or protein expression in adipocytes, and that adipocytes are difficult to work with and are not easily transfected with reagents that work in other cells such as fibroblasts. This is not supported by any reference and would appear to be incorrect in view of the example of Al Hasani, above, who taught electroporation of adipocytes to obtain specific ablation of protein expression. Further the prior art also taught transfection of adipocytes by electroporation, see e.g. Standaert et al (of record) at paragraph bridging pages 30075 and 30076, and paragraph bridging columns 1 and 2 on page 30079. As a result the teachings of the specification in this regard are not persuasive.

Walters taught that the effectiveness of siRNA may depend on transfection technique. Specifically, the results of Walters indicated that siRNAs delivered using cationic lipid transfection techniques were sequestered in the endosome/lysosome pathway in a nonadherent cell line, KAS-6/1 human myeloma cells. See page 417, column 1, first full paragraph. In order to circumvent this problem, Weil used electroporation to deliver the siRNAs because it was known in the prior art that electroporation allowed direct delivery to the cytosol and did not depend on the endosome/lysosome (endocytic) pathway. This provides evidence that it was routine in

the art at the time of the invention to optimize transfection protocols to determine which protocol worked best for a given cell line. It does not provide any evidence that one of ordinary skill in the art would not have had a reasonable expectation of success in obtaining RNAi in adipocytes by electroporating siRNAs. Accordingly, Applicant's reliance on Walters is misplaced.

The Weil reference is relied upon to teach that "the first difficulty in implementing RNA interference with a new cell type is optimizing the transfection process." However, this is essentially an admission that it is simply a matter of optimization, particularly in view of the fact that it was well known in the art that molecules could be electroporated into adipocytes (Al-Hasani, of record, and Zhang, above). Weil also suggested that electroporation of siRNAs can be efficient for nonadherent cells, and stated that the optimal parameters for the electroporation of siRNA differ from those of plasmids, allowing the use of milder conditions that induce less cell toxicity. See abstract. Weil does not provide any evidence that one of ordinary skill in the art would not have had a reasonable expectation of success in obtaining delivering siRNA to adipocytes by electroporation. Instead, Weil provides some motivation for selecting electroporation as a delivery technique for nonadherent cells, stating at page 1247, column 3, that for cells growing in suspension, calcium phosphate precipitation is inappropriate, and liposomes and cationic lipids are unpredictable on new cell lines, whereas electroporation "can, a priori, be adapted to all cell types".

Taken together, the teachings of the specification, Walters, and Weil do not provide evidence that one of ordinary skill in the art would not have had a reasonable expectation of success in combining the cited references.

At page 18, Applicant addresses the rejection over the Paquereau reference. Applicant argues that siRNAs are different entities from the plasmids of Paquereau, as are adipocytes and hepatocytes, such that one of skill in the art would not have had a reasonable expectation of success in utilizing the parameters of Paquereau for delivery of siRNAs. This is unpersuasive because, as discussed above, transfection conditions are routinely optimized by those of skill in the art. Applicant has presented no evidence that electroporation conditions used for plasmids would not function for siRNAs. In fact, Weil suggested only that substitution of siRNA for plasmids simply allows one to use milder conditions that induce less cell toxicity. See abstract. Weil also taught that electroporation can be adapted to all cell types. Because it is routine in the art to optimize transfection parameters, and the prior art taught that electroporation could be adapted to all cell types, there is no reason for one of ordinary skill to lack a reasonable expectation of success. Similarly, since Paquereau exemplified a certain capacitance for mammalian cells, one of ordinary skill would be motivated to use that capacitance as a starting point in the process of optimization.

Applicant addresses the Standaert reference at page 19, arguing essentially that it fails to rectify the deficiencies of the Al Hasani and Clancy references. This is unpersuasive for the reasons set forth above regarding these references.

Applicant addresses the McSwiggen reference at pages 20 and 21. Applicant argues that it fails to rectify the deficiencies of the Al Hasani and Clancy references. This is unpersuasive for the reasons set forth above regarding these references. Applicant also asserts that there is no motivation to combine this reference with Al Hasani and Clancy because McSwiggen is directed to siRNA derivative chemistry and not to the art of glucose transport. This is unpersuasive because McSwiggen was not relied upon to teach glucose transport. Al Hasani taught study of glucose transport by inhibiting the activity of a dynamin gene product through the use of a dominant negative mutant. In view of the teachings of Clancy, one of ordinary skill appreciates that there are several alternative, exchangeable ways to inhibit the activity of a gene product, including inhibiting transcription of the gene, e.g. by siRNA. McSwiggen taught methods of inhibiting gene expression using siRNA, and taught that the stability of siRNA molecules could be enhanced through the use of modified bases. Thus there is clear motivation to modify siRNAs to make them more stable, and it would have been obvious to do so in the method of Al Hasani as modified by Clancy.

For these reasons the rejections are maintained.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Richard Schnizer, whose telephone number is 571-272-0762. The examiner can normally be reached Monday through Friday between the hours of 6:00 AM and 3:30 PM. The examiner is off on alternate Fridays, but is sometimes in the office anyway.

If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, J. Douglas Schultz, can be reached at (571) 272-0763. The official central fax number is 571-273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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